APPENDIX B

Illustrative examples of issued U.S. Patents with claims to ***progeny*** of a cell.

(Downloaded from the CLAIMS®/U.S. Patents Abstracts database from the Dialog® Service of Knight-Ridder Information, Inc. Underlining has been added to relevant claim language.)

United States Patent

Patent Number: US 5436154
Date of Patent: 950725

MONOCLONAL ANTIBODIES AGAINST HUMAN TUMOR NECROSIS FACTOR ALPHA; STABLE HYBRIDOMA CELLS AND ANTIGEN-ANTIBODIES

Inventor(s): Barbanti, Elena, Cologno Monzese, IT; Ghislieri, Marta, Milan , IT; Marcucci, Fabrizio, Legnano, IT; Trizio, Domenico, Cassina Rizzardi, IT
Assignee: Farmitalia Carlo Erba SRL, Milan, IT
Appl. No.: US 806401
Filed: 911213

Related U.S. Application Data

Priority Applic(Ser#,Date): GB 9028123 901228

Int. Cl. A61K-039/395; C07K-015/28; C12N-005/12; C12N-005/20
U.S. Cl. 435240270; 424158100; 424733100; 530387100; 530388230; 530388240
Field of Search 424085800; 424133100; 424158100; 435240270; 530387100; 530388200; 530388230

Primary Examiner - Lacey, David L
Assistant Examiner - Feisee, Lila
Attorney, Agent or Firm - Nikaido, Marmelstein, Murray & Oram

ABSTRACT

A monoclonal antibody is provided which is able to neutralize both human TNF Alpha and TNF Beta , or a binding fragment thereof. A stable hybridoma cell line and progeny thereof are also provided which secrete such a monoclonal antibody. The monoclonal antibody or a fragment thereof may be used to detect the content of human TNF in a sample of body fluid.
005 Claims, 9 Drawing Figures, 5 Drawing Sheets

EXEMPLARY CLAIM

1. A monoclonal antibody which is able to neutralize human Tumor Necrosis Factor (TNF) Alpha , which is able to bind to and precipitate human Tumor Necrosis Factor Alpha to form high molecular weight antigen-antibody complexes, and which is able to neutralize human Tumor Necrosis Factor Beta , wherein said antibody is produced by the hybridoma cell line 78 which is deposited under accession number ECACC 90110707.

NON-EXEMPLARY CLAIMS

2. A monoclonal antibody according to claim 1, wherein the smallest antigen-antibody complexes formed with human TNF Alpha contain about two molecules of said antibody and about one human TNF Alpha molecule and have molecular weight of about 400 kD as measured by gel filtration.
3. A monoclonal antibody according to claim 1 wherein said monoclonal antibody neutralizes trimer human TNF Alpha in vitro at a molar ratio which is about 1.3:1.
4. Hybridoma cell line 78 which is deposited under accession number ECACC 90110707 and progeny thereof.
5. A monoclonal antibody secreted from the hybridoma cell line according to claim 4, or a antigen binding fragment of said antibody

United States Patent

Patent Number: US 5338680
Date of Patent: 940816

NON-PRODUCER CELL LINES TRANSFORMED BY AMV; AVIAN MYELOBLASTUSIS VIRUS

Inventor(s): Moscovici, Carlo, 6816 NW 18th Ave, Gainsville, FL, 32605, (US); Moscovici, M Giovannella, 6816 NW 18th Ave, Gainsville, FL, 32605, (US)
Assignee: Unassigned
Appl. No.: US 109107
Filed: 930819

Related U.S. Application Data

Continuation of(Pat#,Ser#,Date):	ABANDONED		US 529623	900529
	ABANDONED		US 802711	911206
Priority Applic(Ser#,Date):	US 109107	930819		
	US 529623	900529		
	US 802711	911206		

Int. Cl. C12N-005/00; C12N-015/00
U.S. Cl. 435240200; 435172300
Field of Search 435172300; 435240200

Primary Examiner - Ziska, Suzanne E
Attorney, Agent or Firm - Beveridge, DeGrandi, Weilacher & Young

ABSTRACT

AMV-transformed non-producer cells lines which may be grown in chicken serum-free media are disclosed. These cells have been transformed with the avian myeloblastosis virus oncogene, and are capable of being grown without chicken serum.
001 Claims

EXEMPLARY CLAIM

1. A cell line consisting essentially of chicken cells selected from the group consisting of ATCC CRL 10465, and progeny thereof; ATCC CRL 10466, and progeny thereof; ATCC CRL 10467, and progeny thereof; and ATCC CRL 10468, and progeny thereof; which cell line is capable of being cultured and maintained in a culture medium free of chicken serum and free of tryptose phosphate.

United States Patent

Patent Number: US 4624921
Date of Patent: 861125

HUMAN LYMPHOBLASTOLD CELL LINE AND HYBRIDOMAS DERIVED THEREFROM; CULTURES, FUSION

Inventor(s): Larrick, James W, Woodside, CA, (US); Raubitschek, Andrew R, Palo Alto, CA, (US); Truitt, Kenneth E, San Diego, CA, (US)
Assignee: Cetus Corporation, Emeryville, CA
Appl. No.: US 604068
Filed: 840426

Related U.S. Application Data

Priority Applic(Ser#,Date): US 604068 840426

Int. Cl. C12N-015/00; C12N-005/00; C12R-001/91
U.S. Cl. 435172200; 435240270; 435948000; 935100000
Field of Search 435068000; 435172200; 435240000; 435241000; 435948000; 935100000

Primary Examiner - Warren, Charles F
Assistant Examiner - Tarcza, John Edward
Attorney, Agent or Firm - Halluin, Albert P; Hasak, Janet E

ABSTRACT

A 6-thioguanine-resistant subvariant of the EBV-transformed human lymphoblastoid B cell line WI-L2 is described. The subvariant line, designated LTR228, fuses efficiently with human cells. Human X human hybridomas derived from LTR228 that produce monoclonal antibodies against tetanus toxin and blood group A are exemplified.
008 Claims

EXEMPLARY CLAIM

3. A MONOCLONAL ANTIBODY-PRODUCING HUMAN X HUMAN HYBRIDOMA OF: (A) A HUMAN LYMPHOBLASTOID B CELL OF THE LINE LTR228 OR A PROGENY THEREOF; AND (B) AN ANTIBODY-PRODUCING HUMAN CELL.

NON-EXEMPLARY CLAIMS

1. A human lymphoblastoid B cell of the line LTR228 and progeny thereof.
2. A ouabain-resistant progeny of the lymphoblastoid B cell of claim 1.
4. The hybridoma of claim 3 wherein the monoclonal antibody is an anti-tetanus toxoid antibody.
5. A method of producing a human monoclonal antibody comprising: (a) growing the hybridoma of claim 3 in a growth medium; and (b) isolating human monoclonal antibody from the growth medium.
6. A method of making monoclonal antibody-producing human X human hybridomas comprising: (a) fusing lymphoblastoid B cells of the line LTR228 or progeny thereof with human antibody-producing cells in a fusion medium containing a fusogen; (b) separating the cells from the fusion medium; (c) expanding the separated cells; and (d) growing the expanded cells in a medium containing hypoxanthine and azaserine and lacking thymidine whereby said hybridomas are selected.
7. Human X human hybridoma SA13 and progeny thereof.
8. A method for making monoclonal antibody SA13 comprising cultivating hybridoma SA13 in a growth medium and harvesting the antibody from the growth medium

United States Patent

Patent Number: US 4467036
Date of Patent: 840821

BACILLUS THURINGIENSIS CRYSTAL PROTEIN IN ESCHERICHIA COLI; PRODUCTION IN CULTURES OF E. COLI FOR USE AS AN INSECTICIDE

Inventor(s): Schnepf, H Ernest, Seattle, WA, (US); Whiteley, Helen R, Seattle, WA, (US)
Assignee: The Board of Regents of the University of Washington, Seattle , WA
Notice: Portion of the term of this patent, subsequent to 20010515 has been disclaimed
Appl. No.: US 362634
Filed: 820330

Related U.S. Application Data

Cont-in-part of(Pat#,Ser#,Date):	US 4448885 ABANDONED		US 257963	810427
			US 320560	811112
Priority Applic(Ser#,Date):	US 362634	820330		
	US 257963	810427		
	US 320560	811112		

Int. Cl. C12N-001/00; A01N-063/00; C12N-001/20; C12N-015/00; C12P-021/00
U.S. Cl. 435320100; 435069100; 435172300; 435252330; 435832000; 435849000; 935072000
Field of Search .... 424093000; 435068000; 435070000; 435172000; 435172300; 435253000; 435317000 .

Primary Examiner - Wiseman, Thomas
Assistant Examiner - Martinell, James
Attorney, Agent or Firm - Fitch, Even, Tabin & Flannery

ABSTRACT

Expression of the crystal protein of Bacillus thuringiensis in Escherichia coli is described by use of plasmids containing heterologous DNA coding for the crystal protein. Genetically engineered bacterial host strains transformed by the plasmids of the invention express Bacillus thuringiensis crystal proteins without exhibiting the growth phase limitations characteristic of the natural bacterial host species.
003 Claims, 4 Drawing Figures, 2 Drawing Sheets

The United States government has rights in this invention pursuant to a grant awarded by the Department of Health and Human Services.

EXEMPLARY CLAIM

1. A HYBRID RECOMBINANT PLASMID CAPABLE OF REPLICATION IN AN ESCHERICHIA COLI BACTERIAL HOST SPECIES, SAID PLASMID CONTAINING EXPRESSIBLE HETEROLOGOUS DNA CODING FOR A POLYPEPTIDE WHICH HAS THE IMMUNOLOGICAL PROPERTIES OF THE CRYSTAL PROTEIN OF BACILLUS THURINGIENSIS, SAID PLASMID FURTHER CONTAINING EXPRESSIBLE HETEROLOGOUS DNA HAVING A DNA PORTION DERIVED FROM PLASMIDS OF BACILLUS THURINGIENSIS HAVING A MOLECULAR MASS GREATER THAN 10 X 106 MR, SAID BACILLUS THURINGIENSIS DERIVED DNA PORTION BEING IDENTIFIABLE WITH A PVU II-C DNA FRAGMENT PROBE, SAID HYBRID RECOMBINANT PLASMID FURTHER COMPRISING AN EXPRESSION MECHANISM FOR SAID EXPRESSIBLE HETEROLOGOUS DNA WHICH IS RECOGNIZED BY THE HOST SPECIES' SYSTEM.

NON-EXEMPLARY CLAIMS

2. A hybrid recombinant plasmid capable of replication in an Escherichia coli bacterial host species, said plasmid containing expressible heterologous DNA coding for a polypeptide which has the immunological properties of the crystal protein of Bacillus thuringiensis, said

plasmid further containing expressible heterologous DNA having a DNA portion derived from plasmids of Bacillus thuringiensis having a molecular mass greater than 10 X 106 Mr, said Bacillus thuringiensis derived DNA portion further being identifiable with a Pvu II-C DNA fragment probe, said Bacillus thuringiensis derived DNA portion further being selected from the group consisting of Bacillus thuringiensis subspecies tolworthi; Bacillus thuringiensis subspecies darmstadiesis; Bacillus thuringiensis subspecies sotto; Bacillus thuringiensis subspecies thuringiensis; Bacillus thuringiensis subspecies thuringiensis, strain HD-290; Bacillus thuringiensis subspecies thuringiensis, strain HD-120; Bacillus thuringiensis subspecies thuringiensis, strain HD-2; Bacillus thuringiensis subspecies kurstaki, strain HD-244; Bacillus thuringiensis subspecies kurstaki, strain HD-73; Bacillus thuringiensis subspecies kurstaki, strain HD-1; Bacillus thuringiensis subspecies alesti, strain HD-4; Bacillus thuringiensis subspecies toumanoffi, strain F-9; Bacillus thuringiensis subspecies galleriae, strain HD-8; Bacillus thuringiensis subspecies wuhnanesis, strain F-6 and Bacillus thuringiensis subspecies morrisoni, strain F-5; said hybrid recombinant plasmid further including an expression mechanism for said heterologous DNA which is recognized by the host species' system.

3. Hybrid recombinant plasmids having the Bacillus thuringiensis crystal protein coding characteristics of plasmids: pES1, as carried in Escherichia coli strain ES12, (ATCC Number 31995); pJWK20, as carried in Escherichia coli strain JWKI (ATCC Number 31997); and pJWK18, as carried in Escherichia coli strain JWKII, (ATCC Number 31998), and their progeny resulting from normal cell division of the parental bacterial cells or plasmids.

United States Patent

Patent Number: US 5489744
Date of Patent: 960206

INBRED CORN LINE 4P33339

Inventor(s): Dallas, Lyndall W, Lincoln, IL, (US)
Assignee: Agrigenetrics, LP, Madison, WI
Appl. No.: US 95286
Filed: 930721

Related U.S. Application Data

Priority Applic(Ser#,Date): US 95286 930721

Int. Cl. A01H-001/02; A01H-004/00; A01H-005/00; C12N-005/04
U.S. Cl. 800200000; 047DIG001; 047058000; 435240400; 435240490; 435240500; 800DIG056; 800250000
Field of Search 047058010; 047058030; 435172100; 435172300; 435240100; 435240400; 435240470; 435240490; 435240500; 800DIG052; 800DIG056; 800200000; 800205000; 800235000; 800250000

Primary Examiner - Benzion, Gary
Assistant Examiner - Veitenheimer, Erich E
Attorney, Agent or Firm - Saliwanchik & Saliwanchik

ABSTRACT

According to the invention, there is provided an inbred corn line, designated 4P33339. Further provided are the plants and seeds of inbred corn line 4P33339, and hybrids produced using 4P33339 inbred line as one parent crossed with a distinct inbred corn line.
007 Claims

EXEMPLARY CLAIM

1. Inbred corn seed designated 4P33339 and having ATCC Deposit No. 97177.

NON-EXEMPLARY CLAIMS

2. A corn plant produced by the seed of claim 1 and its parts.
3. A tissue culture of regenerable cells of the corn plant designated 4P33339 and having ATCC Deposit No. 97177.
4. An F1 hybrid corn plant having 4P33339 as a parent, wherein 4P33339 has ATCC Deposit No. 97177.
5. Corn seed which results from a cross of the corn plant designated 4P33339 with another corn plant that is not 4P33339, wherein 4P33339 has ATCC Deposit No. 97177.
6. A tissue culture of regenerable cells of an F1 hybrid corn plant which is progeny of 4P33339, wherein 4P33339 has ATCC Deposit No. 97177.
7. Corn seed which, when grown under suitable conditions, gives rise to the F1 hybrid corn plant of claim 4

United States Patent

Patent Number: US 5502271
Date of Patent: 960326

MAIZE RESISTANT TO ARYLOXYPHENOXYALKANECARBOXYLIC ACID HERBICIDES

Inventor(s): Donn, Gunter, Hofheim am Taunus, DE
Assignee: Hoechst Aktiengesellschaft, Frankfurt am Main, DE
Appl. No.: US 464295
Filed: 950605

Related U.S. Application Data

Continuation of(Pat#,Ser#,Date):ABANDONED US 70430 930608
ABANDONED US 245064 940517
Priority Applic(Ser#,Date): EP 91103765 910312

Int. Cl. A01H-001/04; A01H-001/06; C12N-015/01; C12N-005/04
U.S. Cl. 800200000; 047058000; 435172100; 435240400; 435240470; 435240480; 435240490; 435240500; 800DIG056; 800235000; 800250000
Field of Search 047058000; 435172100; 435172300; 435240400; 435240480; 435240500; 800DIG052; 800DIG056; 800200000; 800235000; 800250000

Primary Examiner - Fox, David T
Assistant Examiner - Veitenheimer, Erich E
Attorney, Agent or Firm - Curtis, Morris & Safford

ABSTRACT

By selection for resistance to aryloxyphenoxyalkanecarboxylic acid herbicides, herbicide-tolerant maize cell lines, calli and plants regenerated therefrom can be obtained which pass this herbicide tolerance on to their progeny in a stable manner.
015 Claims

EXEMPLARY CLAIM

2. A maize plant regenerated from auxin-autotrophic maize cells, maize protoplasts, maize cell cultures and maize calli as well as progeny thereof which is resistant to conventional application concentrations of aryloxyphenoxyalkanecarboxylic acid herbicides, and the progeny of such a maize plant.

NON-EXEMPLARY CLAIMS

1. An auxin-autotrophic maize cell, maize protoplast, maize cell culture or maize callus which is resistant to aryloxyphenoxyalkanecarboxylic acid herbicides and the progeny of such a maize cell.
3. A maize cell, maize protoplast, maize cell culture or maize callus as well as progeny thereof as claimed in claim 1 and a plant as claimed in claim 2 which are resistant to a further herbicide.
4. A method for controlling grasses comprising applying to said grasses an aryloxyphenoxyalkanecarboxylic acid herbicide, alone or in combination with each another, aryloxyphenoxyalkanecarboxylic acid herbicide, wherein the grasses are in cultures of maize plants regenerated from auxin-autotrophic maize cells, maize protoplasts from such cells, maize cell cultures from such cells, maize calli from such cells, or progeny thereof, which is resistant to conventional application concentrations of aryloxyphenoxyalkanecarboxylic acid herbicides.
5. A maize plant as claimed in claim 2, wherein fenoxaprop-ethyl is used as the herbicide.
6. A maize plant as claimed in claim 2, which is resistant to fenoxaprop-ethyl at application rates of between 20 and 200 g of a.i./ha.
7. A maize plant as claimed in claim 2, which is resistant to fenoxaprop-ethyl at application rates of between 30 and 150 g of a.i./ha.
8. A maize plant as claimed in claim 2, which is resistant to

fenoxaprop-ethyl at application rates of between 30 and 90 g of a.i./ha.

9. A maize plant as claimed in claim 2, which is resistant to aryloxyphenoxyalkanecarboxylic acid herbicides and glutamine synthetase inhibitors.
10. A maize plant as claimed in claim 2, which is resistant to fenoxaprop-ethyl and phosphinothricin or bialaphos.
11. A maize cell, maize protoplast, maize cell culture or maize callus as claimed in claim 1, which is resistant to fenoxaprop-ethyl.
12. A maize cell, maize protoplast, maize cell culture or maize callus as claimed in claim 1, which is resistant to 1 X 10-6M to 1 X 10-3M fenoxapropethyl.
13. A maize cell, maize protoplast, maize cell culture or maize callus as claimed in claim 1, which is resistant to 5 X 10-6M to 5 X 10-3M fenoxapropethyl.
14. A method for the production of herbicide-resistant maize cell lines, which comprises exposing auxinautotrophic maize cell lines stepwise to increasing concentrations of aryloxyphenoxyalkanecarboxylic acid herbicides and propagating the mutants which survive in each case.
15. A method for the production of herbicide-resistant maize cell lines, which comprises exposing auxinautotrophic maize cell lines to aryloxyphenoxyalkanecarboxylic acid herbicides at increasing concentrations of between 1 X 10-6M and 1 X 10-3M and propagating the mutants which survive in each case

United States Patent

Patent Number: US 5462862
Date of Patent: 951031

METHOD AND COMPOSITIONS FOR ENHANCING PRODUCTION OF SECONDARY METABOLITES USING CLUSTERED BIOSYNTHETIC GENES; ANTIBIOTICS FROM MICROORGANISMS

Inventor(s): Alvarez, Emilio, Sevilla, ES; Barredo, Jose L, Trespaderne, ES; Diez, Bruno, Sebastian, ES; Esmahan, Christina, Bilbao, ES; Groenen, Martinus A M, Zetten, NL; Gutierrez, Santiago, Leon, ES; Koekman, Bertus P, Schipluiden, NL; Martin, Juan F, Leon, ES; Van Der Voort, Lucia H M, Delft, NL; Van Solingen, Pieter, Naaldwijk, NL; Veenstra, Annemarie E, Vennep, NL
Assignee: Gist-brocades NV, Delft, NL
Appl. No.: US 8688
Filed: 930125

Related U.S. Application Data
Continuation of(Pat#,Ser#,Date):ABANDONED US 392119 890810
Priority Applic(Ser#,Date): EP 88201714 880811
EP 89201044 890421

Int. Cl. C12P-021/06; C07H-017/00; C12N-001/20; C12N-015/00
U.S. Cl. 435069100; 435172300; 435252100; 435252300; 435320100; 536023100; 536023400; 536023700
Field of Search 435006000; 435069100; 435070100; 435172100; 435172200; 435172300; 435235000; 435240200; 435252100; 435252300; 435320100; 530350000; 530371000; 536023100; 536023400; 536023700

Primary Examiner - Draper, Garnette D
Assistant Examiner - Wang, Gian P
Attorney, Agent or Firm - Rae-Venter, Barbara

ABSTRACT

Clustered antibiotic biosynthetic genes are employed for improvement of production of the antibiotic in microorganisms and for the isolation of other genes involved in the biosynthesis of the antibiotic. The invention is exemplified with improved production of penicillin in Penicillium chrysogenum, with the isolation of another clustered biosynthetic gene(s) and with the expression of penicillin biosynthetic genes in Acremonium chrysogenum.
017 Claims, 13 Drawing Figures, 13 Drawing Sheets

EXEMPLARY CLAIM

D R A W I N G

1. A DNA construct comprising at least two genes selected from the group consisting of genes encoding isopenicillin N synthetase, acyltransferase and ACV synthetase.

NON-EXEMPLARY CLAIMS

2. A DNA construct according to claim 1 comprising a gene combination selected from the group consisting of (a) a combination of the genes encoding isopenicllin N synthetase and acyltransferase, (b) a combination of the genes encoding isopenicillin N synthetase and ACV synthetase, (c) a combination of the genes encoding ACV synthetase and acyltransferase, and (d) a combination of the genes encoding ACV synthetase, acyltransferase, and isopenicillin N synthetase.
3. A DNA construct according to any one of claims 1, and 2, wherein at least one of said genes is capable of complementing a Beta -lactam non-producing mutation.
4. A vector comprising a DNA construct selected from the group consisting of pGJ02 A, pGJ02 B and HM 193.
5. A vector comprising a DNA construct according to any one of claims 1, 2 or 4, comprising at least one member selected from the group consisting

of a marker for selection in a host producing said Beta -lactam antibiotic and a sequence for enhancing transformation efficiency of said vector in said host.

6. A transformed host cell comprising a DNA construct according to any one of claims 1 and 3, or a vector according to claim 4.
7. A transformed host cell comprising a DNA construct according to any one of claims 1 and 3, or a vector according to claim 4 obtained by a strain improvement procedure selected from the group consisting of protoplast fusion, mass mating and mutation.
8. A transformed host comprising a vector according to claim 5.
9. A transformed host cell comprising a DNA construct according to any one of claims 1 and 3, or a vector according to claim 4 obtained by a strain improvement procedure selected from the group consisting of protoplast fusion, mass mating and mutation, wherein said host cell is selected from the group consisting of Penicillium, Aspergillus, Acremonium and Actinomycetes.
10. A transformed host according to claim 9 wherein said Penicillium is Penicillium chrysogenum.
11. A transformed host cell comprising a vector comprising a DNA construct according to any one of claims 1 and 3, or a vector according to claim 4 comprising at least one member selected from the group consisting of a marker for selection in a host cell producing said Beta -lactam antibiotic and a sequence for enhancing transformation efficiency of said vector in said host cell, wherein said transformed host cell is obtained by a strain improvement procedure selected from the group consisting of protoplast fusion, mass mating and mutation.
12. Progeny of a transformed host cell according to claim 6.
13. Progeny of a transformed host cell according to claim 7.
14. Progeny of a transformed host cell according to claim 9.
15. Progeny of a transformed host cell according to claim 11.
16. A method for obtaining or enhancing the production of a Beta -lactam antibiotic in a microbial host comprising: preparing a DNA construct according to any one of claims 1, 2 or 4; transforming a candidate host with said DNA construct; obtaining clones of the resulting transformants; and identifying clones having enhanced production of said Beta -lactam antibiotic as compared to an untransformed candidate host.
17. A method for enhancing the production of a Beta -lactam antibiotic comprising: growing a host cell comprising an extra copy of at least two genes selected from the group consisting of genes encoding isopenicillin N synthetase, acyltransferase and ACV synthetase, wherein said host cell or an ancestor of said host cell is a transformant, resulting in enhanced production of said antibiotic; and isolating the resulting antibiotic product

United States Patent

Patent Number: US 5324643
Date of Patent: 940628

METHOD OF CONFERRING RESISTANCE TO RETROVIRAL INFECTION; BY INTERFERING WITH ONE OR MORE OF THE INFECTION PROCESSES INCLUDING RETROVIRAL REPLICATION AND ASSEMBLY INTO INFECTIVE VIRAL PARTICLES

Inventor(s): Greatbatch, Wilson, Akron, NY, (US); Sanford, John C, Geneva, NY, (US)
Assignee: Greatbatch Gen-Aid, Ltd, Clarence, NY
Appl. No.: US 739718
Filed: 910729

Related U.S. Application Data

Cont-in-part of(Pat#,Ser#,Date):ABANDONED US 156188 880216
Priority Applic(Ser#,Date): US 739718 910729
US 156188 880216

Int. Cl. C12N-015/11; C07H-021/02; C07H-021/04; C12N-005/10
U.S. Cl. 435091320; 435091100; 435091300; 435172300; 435240100; 435240200; 536023100; 935003000; 935006000; 935034000; 935070000
Field of Search 435091000; 435091100; 435091300; 435091320; 435172300; 435240100; 435240200; 536023100; 536027000

Primary Examiner - Stone, Jacqueline
Assistant Examiner - Railey, II, Johnny F
Attorney, Agent or Firm - Hodgson, Russ, Andrews, Woods & Goodyear

ABSTRACT

In accordance with the present invention, disclosed is a method of conferring, upon a host cell, resistance to retroviral infection by interfering with one or more of the infection processes including retroviral replication and assembly into infective viral particles. The method involves introducing a vector into a host cell, wherein the vector comprises a polynucleotide which directs transcription, within the host cell, of RNA which is a) complementary or homologous, depending on the target region, to a nucleic acid sequence within one or more regions of the genome of the retrovirus; and b) is effective in inhibiting retroviral replication and/or interfering with assembly into viral particles when the host cell is infected. Also disclosed is a method of treatment using cells upon which resistance to infection has been conferred.

034 Claims, 36 Drawing Figures, 19 Drawing Sheets

EXEMPLARY CLAIM

1. A method of conferring resistance to retroviral infection upon a host cell by inhibiting in the infection process at least one step of the process selected from the group consisting of retroviral replication, reverse transcription, and translation, said method comprising: Introduction into said host cell in vitro of a vector comprising a polynucleotide which is transcribed to RNA, within said host cell, said RNA is complementary to a nucleic acid sequence within at least one region within the genome of said retrovirus, wherein said region is an essential hybridization site within the retroviral genome selected from the group consisting of the 3'R-region, the primer binding (PBS) region, the AUG start codon region, and RNA splice sites; and wherein said retrovirus is feline leukemia virus (FeLV).

NON-EXEMPLARY CLAIMS

2. The method of claim 1, wherein said polynucleotide is a synthetic polynucleotide.
3. The method of claim 1, wherein said polynucleotide is DNA.
4. The method of claim 1, wherein said vector is selected from the group

consisting of a viral vector, a retroviral vector and a plasmid.

5. The method of claim 4, wherein said vector is a plasmid.
6. The method of claim 1, wherein said polynucleotide directs transcription of a single RNA which is complementary to the multiple hybridization sites within the retrovirus genome.
7. The method of claim 4, wherein said vector further comprises a first promoter which controls transcription of said RNA within said host cell.
8. The method of claim 4, wherein said vector further comprises a first terminator which controls termination of transcription of said RNA within said host cell.
9. The method of claim 4, wherein said vector further comprises a marker for selection of transformed cells.
10. The method of claim 7, wherein said polynucleotide further comprises a second promoter which controls transcription of said RNA within said host cell.
11. The method of claim 10, wherein said promoter is RNA Polymerase III promoter.
12. The method of claim 8, wherein said polynucleotide further comprises a second terminator which controls termination of transcription of said RNA within said host cell.
13. The method of claim 12, wherein said terminator is a RNA Polymerase III terminator sequence.
14. A nucleic acid construct conferring resistance to retroviral infection upon a host cell by inhibiting in the infection process at least one step of the process selected from the group consisting of retroviral replication, reverse transcription, and translation, said construct comprising a polynucleotide which when introduced by a vector into the host cell in vitro results in transcription of the polynucleotide into RNA complementary to the nucleic acid sequences within multiple regions within the genome of said retrovirus, wherein said regions are essential hybridization sites within the retroviral genome consisting of the 3'R-region, the primer binding (PBS) region, the AUG start codon region, and RNA splice sties of said retrovirus; and wherein said retrovirus is FeLV 15. The nucleic acid construct of claim 14, wherein said polynucleotide is a synthetic polynucleotide.
16. The nucleic acid construct of claim 14, wherein said polynucleotide is DNA.
17. The nucleic acid construct of claim 14, wherein said vector is selected from the group consisting of a viral vector, a retroviral vector and a plasmid.
18. The nucleic acid construct of claim 17, wherein said vector is a plasmid.
19. The nucleic acid construct of claim 17, wherein said vector further comprises a first promoter which controls transcription of said RNA within said host cell, and a first terminator which controls termination of said transcription.
20. The nucleic acid construct of claim 17, wherein said vector further comprises a marker for selection of transformed cells.
21. The nucleic acid construct of claim 19, wherein said polynucleotide comprises a second promoter which controls transcription of said RNA within said host cell.
22. The nucleic acid construct of claim 21, wherein said promoter is RNA Polymerase III promoter.
23. The nucleic acid construct of claim 19, wherein said polynucleotide comprises a second terminator which controls termination of transcription of said RNA within said host cell.
24. The nucleic acid construct of claim 23, wherein said terminator is a RNA Polymerase III terminator sequence.
25. An RNA molecule, produced from the transcription of a polynucleotide of a vector which has been introduced into a host cell in vitro, said RNA molecule (a) confers resistance to retroviral infection upon a host cell by inhibiting in the infection process at least one step of the process selected from the group consisting of retroviral replication, reverse transcription, and translation; and (b) is complementary to the nucleic acid sequences within multiple regions within the genome of said retrovirus, wherein said regions are essential hybridization sties within the retroviral genome consisting of the 3'R-region, the primer binding (PBS) region, the AUG start codon region, and RNA splice sites of said retrovirus, and wherein said retrovirus is FeLV.
26. The RNA molecule of claim 25, wherein said vector further comprises a first promoter which controls transcription of said RNA within said host cell.

27. The RNA molecule of claim 25, wherein said vector further comprises a first terminator which controls termination of transcription of said RNA within said host cell.
28. The RNA molecule of claim 25, wherein said vector further comprises a marker for selection of transformed cells.
29. The RNA molecule of claim 26, wherein said polynucleotide comprises a second promoter which controls transcription of said RNA within said host cell.
30. The RNA molecule of claim 27, wherein said promoter is RNA Polymerase III promoter.
31. The RNA molecule of claim 27, wherein said polynucleotide comprises a second terminator which controls termination of transcription of said RNA within said host cell.
32. The RNA molecule of claim 31, wherein said terminator is a RNA Polymerase III terminator sequence.
33. A cell modified by the method of claim 1.
34. Progeny of the cell of claim 33.

United States Patent

Patent Number: US 5252466
Date of Patent: 931012

FUSION PROTEINS HAVING A SITE FOR IN VIVO POST-TRANSLATION MODIFICATION AND METHODS OF MAKING AND PURIFYING THEM; HYBRIDS

Inventor(s): Cronan, Jr, John E, Urbana, IL, (US)
Assignee: Biotechnology Research and Development Corporation, Peoria, IL; Board of Trustees of the University of Illinois, Champaign, IL
Appl. No.: US 525568
Filed: 900518

Related U.S. Application Data

Cont-in-part of(Pat#,Ser#,Date):ABANDONED US 354266 890519
Priority Applic(Ser#,Date): US 525568 900518
US 354266 890519

Int. Cl. C12N-015/62; C12N-015/63
U.S. Cl. 435069700; 435252300; 435320100; 530350000
Field of Search 435069700; 435252300; 435320100; 530350000; 536027000

Primary Examiner - Hill, Jr, Robert J
Assistant Examiner - Ulm, John D
Attorney, Agent or Firm - Willian Brinks Olds Hofer Gilson & Lione

ABSTRACT

A hybrid DNA sequence encoding a fusion protein comprising: a first DNA sequence which encodes an amino acid sequence that allows for post-translation modification of the fusion protein; and a second DNA sequence joined end to end with the first DNA sequence and in the same reading frame, the second DNA sequence encoding a selected protein or polypeptide. Also, a vector comprising this hybrid DNA sequence, a host transformed with the vector, and a method of producing the fusion protein comprising culturing the transformed host under conditions permitting expression of the fusion protein. Further, a fusion protein comprising a selected protein or polypeptide and an amino acid sequence that allows for post-translation modification of the fusion protein. Finally, a method of isolating the modified fusion protein from a mixture of materials comprising: providing a binding partner that binds to the fusion protein only after it has been modified; contacting the modified fusion protein with the binding partner under conditions permitting binding; separating the modified fusion protein bound to the binding partner from the unbound materials in the mixture; and eluting the modified fusion protein.
009 Claims, 36 Drawing Figures, 33 Drawing Sheets

The invention described herein was made in the course of work partially funded by Grant No. 2 RO1 AI15650 from the National Institutes of Health, U.S. Department of Health and Human Services. The U.S. government may have rights in this invention.

EXEMPLARY CLAIM

1. A transformed host cell into which DNA has been introduced, or progeny of said transformed host cell , the introduced DNA comprising: (a) DNA coding for a fusion protein comprising: (i) a first DNA sequence which codes for a protein or polypeptide having an amino acid sequence that allows for posttranslation biotination of the fusion protein; and (ii) a second DNA sequences joined end to end with the first DNA sequence and in the same reading frame, the second DNA sequence encoding a selected protein or polypeptide; and (b) DNA coding for biotin ligase; the DNA coding for the fusion protein and the DNA coding for biotin ligase being operatively linked to expression control sequences.

NON-EXEMPLARY CLAIMS

2. The host cell of claim 1 wherein the DNA coding for the fusion protein further comprises a third DNA sequence which codes for a cleavage site, the third DNA sequence being located between the first and second DNA sequences, all three DNA sequences being in the same reading frame.
3. The host cell of claim 11 wherein the first DNA sequence codes for the 1.3S subunit of Propionibacterium shermanii transcarboxylase, tomato biotin protein, the alpha subunit of Klebsiella pneumoniae oxalacetate decarboxylase, Escherichia coli biotin carboxyl carrier protein or fragments of these proteins that allow for post-translation biotination of the fusion protein.
4. The host cell of claim 3 wherein the first DNA sequence codes for the final 75 amino acids of the 1.3S subunit of Propionibacterium shermanii transcarboxylase.
5. The host cell of claim 3 wherein the first DNA sequence codes for the alpha subunit of Klebsiella pneumoniae oxalacetate decarboxylase, or fragments thereof that allow for post-translation biotination of the fusion protein.
6. The host cell of claim 5 wherein the first DNA sequence codes for the potion of the alpha subunit of Klebsiella pneumoniae oxalacetate decarboxylase having the following amino acid sequence:

D R A W I N G

7. A method of producing a fusion protein comprising culturing the transformed host cell of any one of claims 1-6 under conditions permitting expression of the fusion protein and the biotin ligase and permitting biotination of the fusion protein.
8. The host cell of any one of claims 1-6 wherein the DNA coding for the fusion protein further comprises an additional DNA sequence which codes for a signal or signal-leader peptide, or fragment thereof, the additional DNA sequence being located upstream of the first and second DNA sequences and being operatively linked to them so as to provide for secretion of the fusion protein.
9. A method of producing a fusion protein comprising culturing the transformed host cell of claim 8 under conditions permitting expression of the fusion protein and the biotin ligase and permitting biotination of the fusion protein.